

# Establishment and characterization of a PDX based preclinical platform for checkpoint inhibitor testing and development of translational biomarker

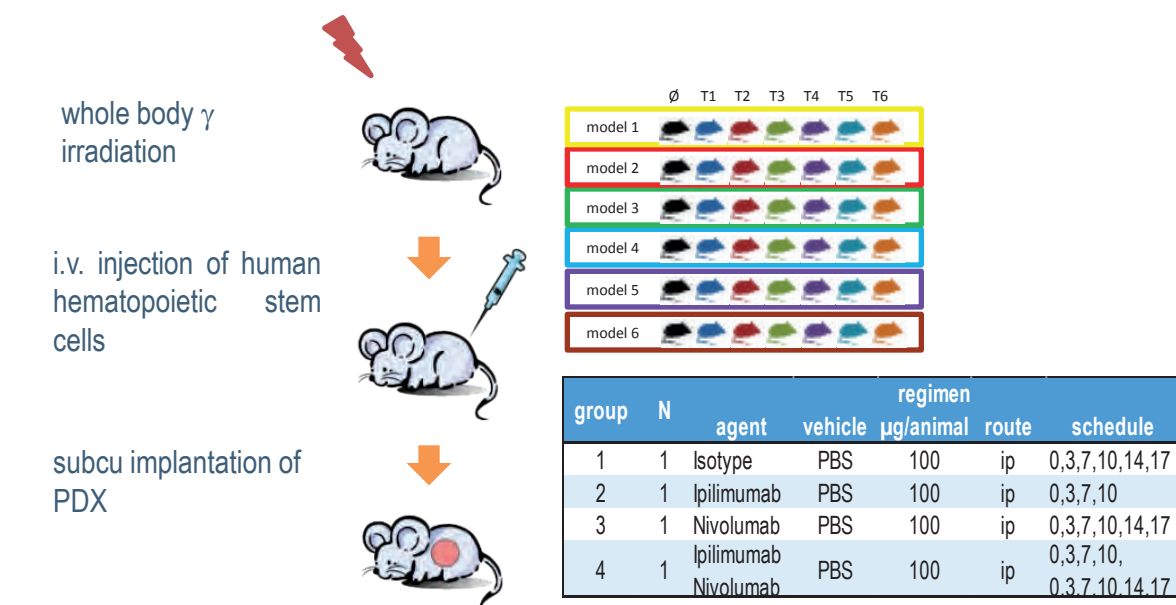
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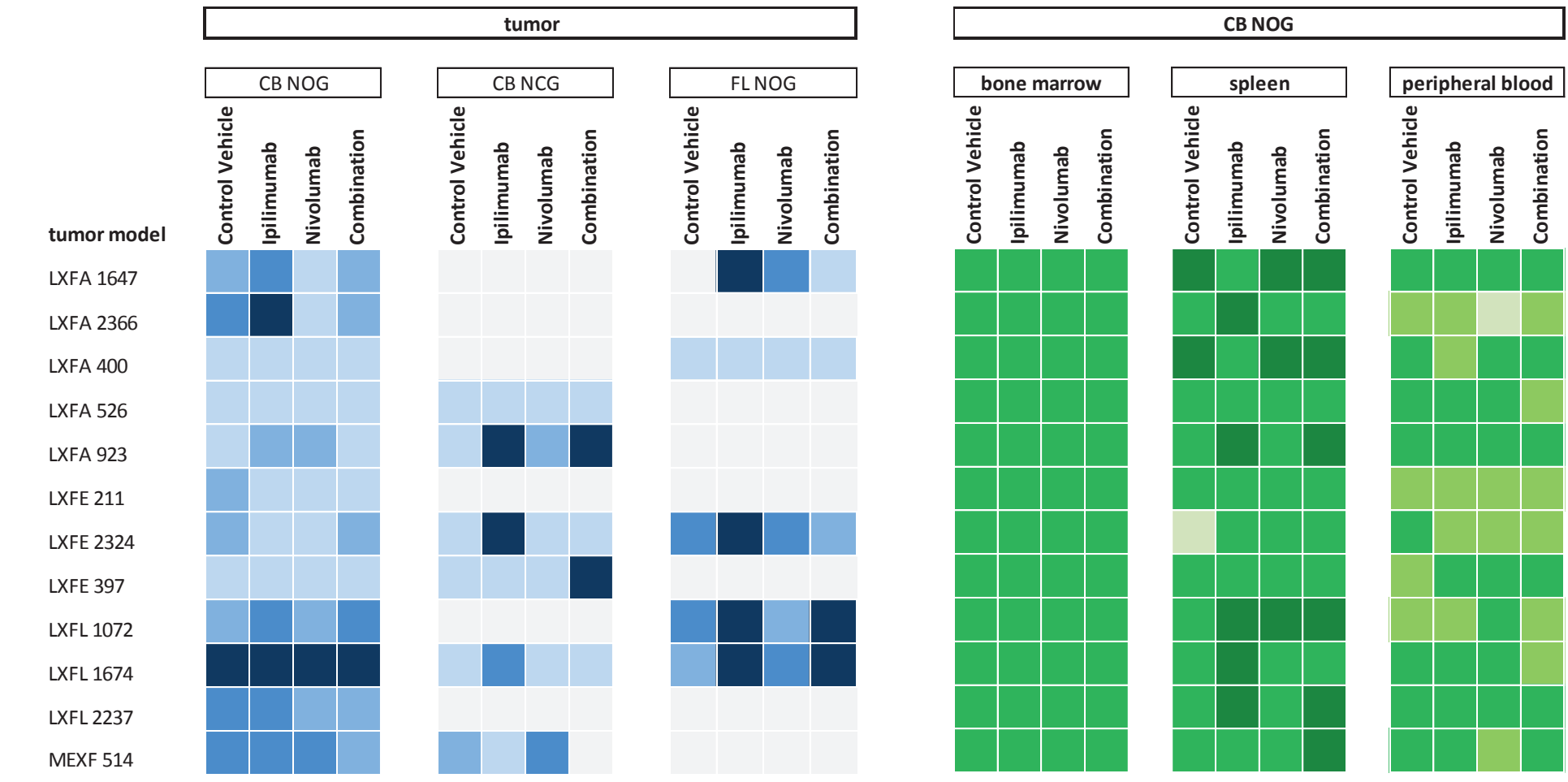
## 1 INTRODUCTION

Patient-derived tumor xenografts (PDX) play a major role in the development of new cancer therapies. However, one major drawback of PDX is the lack of an immunological competent host. To overcome this hurdle, the use of humanized mouse models is gaining more and more importance. The current project aims to establish a drug screening workflow bridging between innovative mouse models and biomarker development. A total of 69 NOG (NOD/Shi-scid/IL-2R $\gamma$  null) and 25 NCG (NOD-Prkdc<sup>em26Cd52</sup>Il2rgem26Cd22/NjuCrI) mice were engrafted with CD34+ hematopoietic stem cells. Thereafter, tumor material from 11 different lung cancer and one melanoma patient derived xenograft models was implanted subcutaneously. Individual mice were treated with  $\alpha$ -CTLA-4,  $\alpha$ -PD-1 or the combination thereof. With n=1 per treatment arm and model the study design followed the screening approach of the single mouse trial (SMT). Infiltration of human immune cells and PDL-1 expression was detected by flow cytometry (FC) and immunohistochemistry (IHC) in hematopoietic organs and tumor tissue. A computerized analysis for digitized whole-slide images of the samples was used to quantify the results using color classification and morphological image processing techniques.

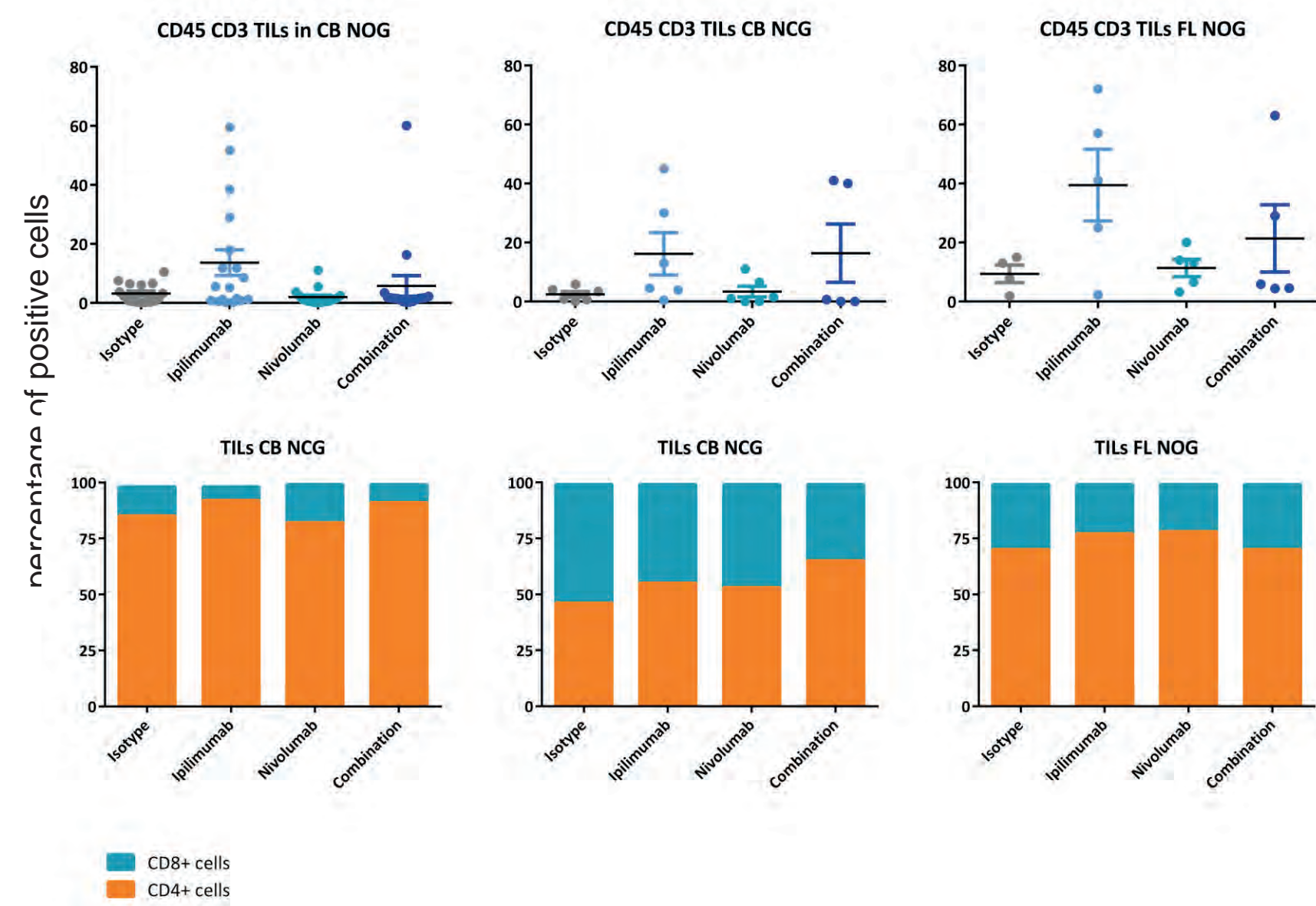
**Fig. 1 Study design:** NOG mice were irradiated and engrafted with human hematopoietic stem cells either from cord blood (CB) or fetal liver (FL). Twelve PDX models (11 NSCLC and one melanoma) were implanted subcutaneous and individual mice assigned to one of the depicted treatment arms: Isotype, Ipilimumab, Nivolumab or the combination thereof.



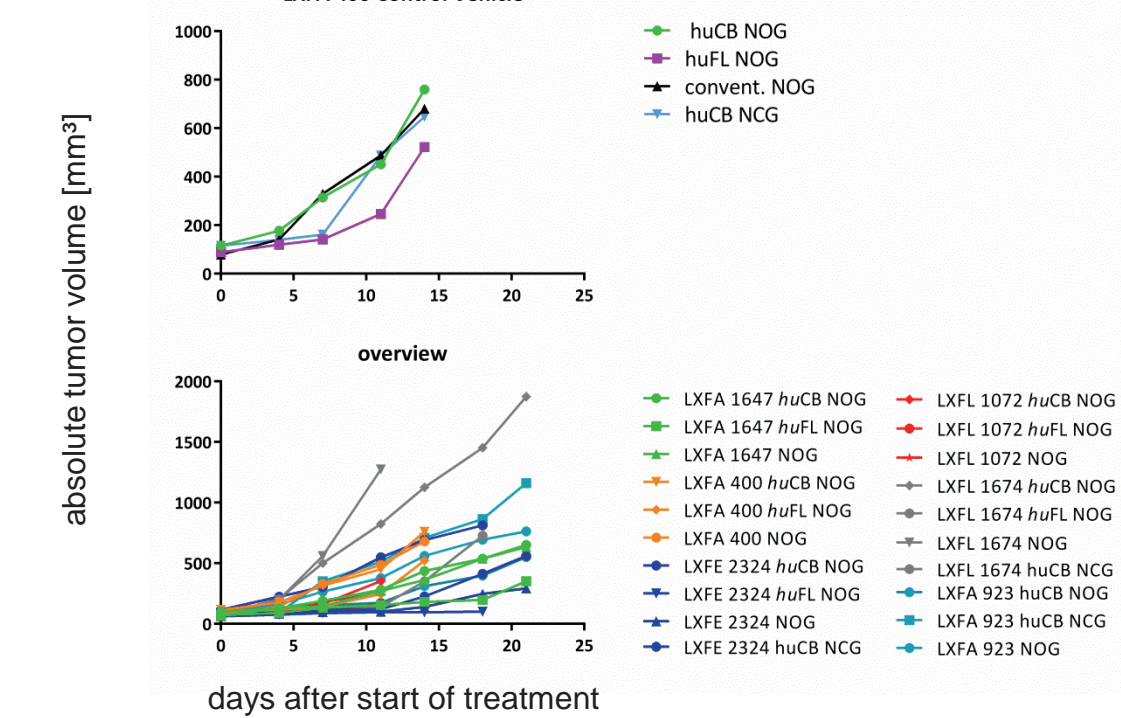
**Fig. 4 Infiltration of tumor and hematopoietic organs of hu-mice bearing NSCLC PDX under treatment with checkpoint inhibitors.** Numbers of huCD45+ cells were significantly increased specifically by Ipilimumab treatment. Bone marrow and spleen of humanized mice showed high (>25%) amounts of huCD45 cells independent of treatment regimen, PDX model or host.



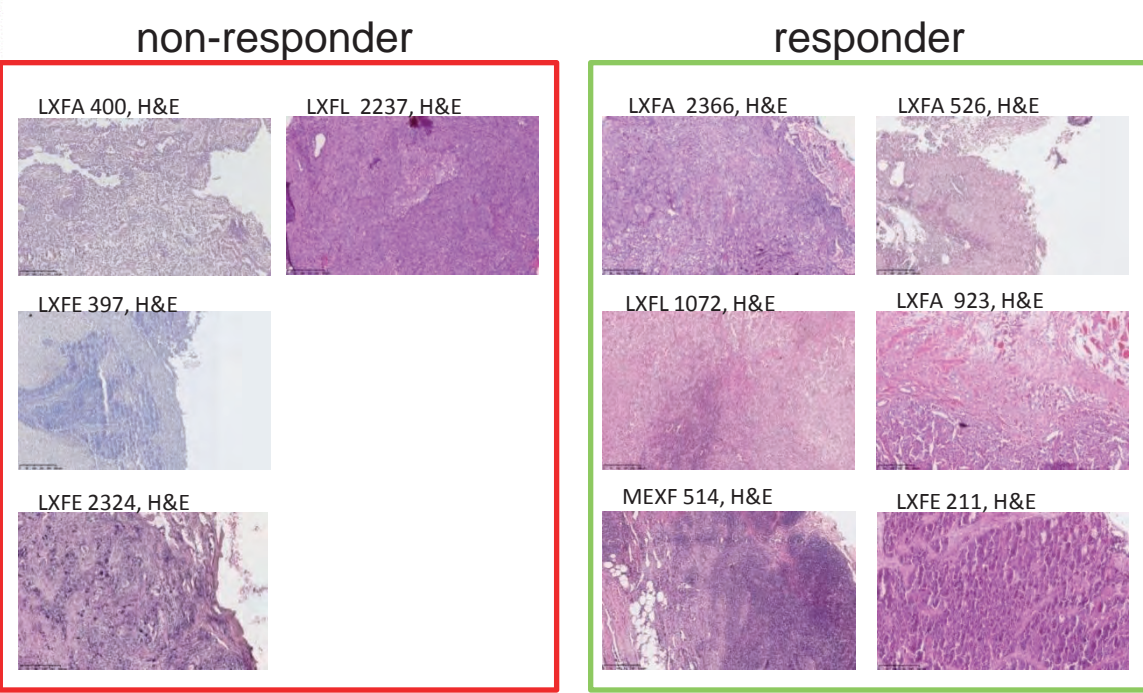
**Fig. 5 Infiltration of tumor of hu-mice bearing NSCLC PDX under treatment with checkpoint inhibitors.** The number of CD45+/CD3+ cells in tumor tissue was markedly increased in mice treated with Ipilimumab. The CD4/CD8 ratio differs in the different murine hosts. Nevertheless, a slight increase under treatment could be observed in most settings.



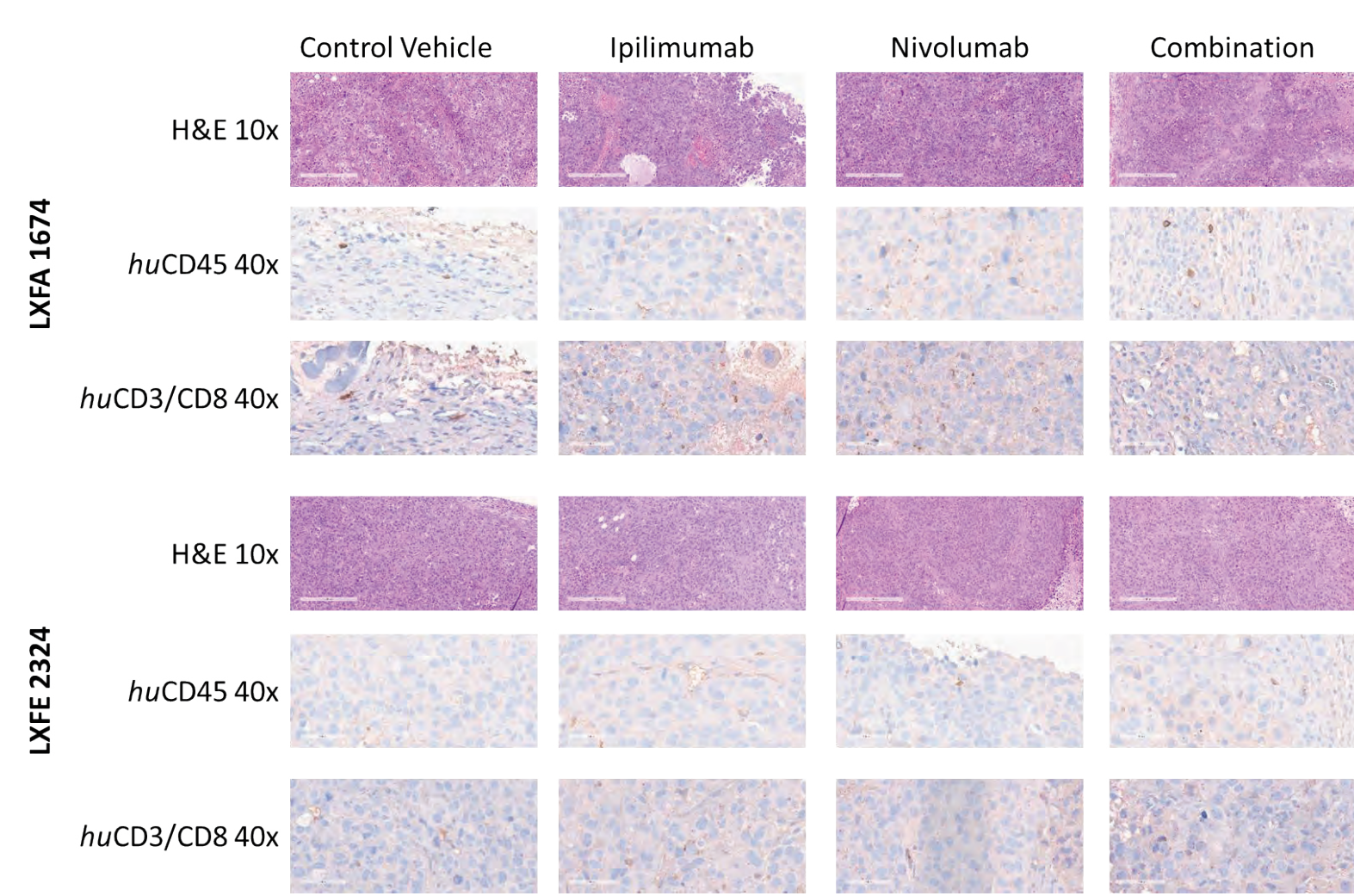
**Fig. 2 growth behavior of PDX models.** The investigated PDX models showed a distinct growth behavior independent of the host immune status (CB vs FL; NOG vs NCG) and analogous to the corresponding features depicted in conventional NOG.



**Fig. 6 H&E stains of tumor tissue from donor patients of selected PDX models.** Tumor models with high tumor infiltrating lymphocyte (TIL) rates in the donor patient material tended to be more sensitive towards checkpoint inhibitor treatment as models with low rates. When dividing individual PDX models into responders and non-responders (cut-off: 30% T/C value). The two groups depicted marked differences in the TIL infiltration rate of donor tissue.

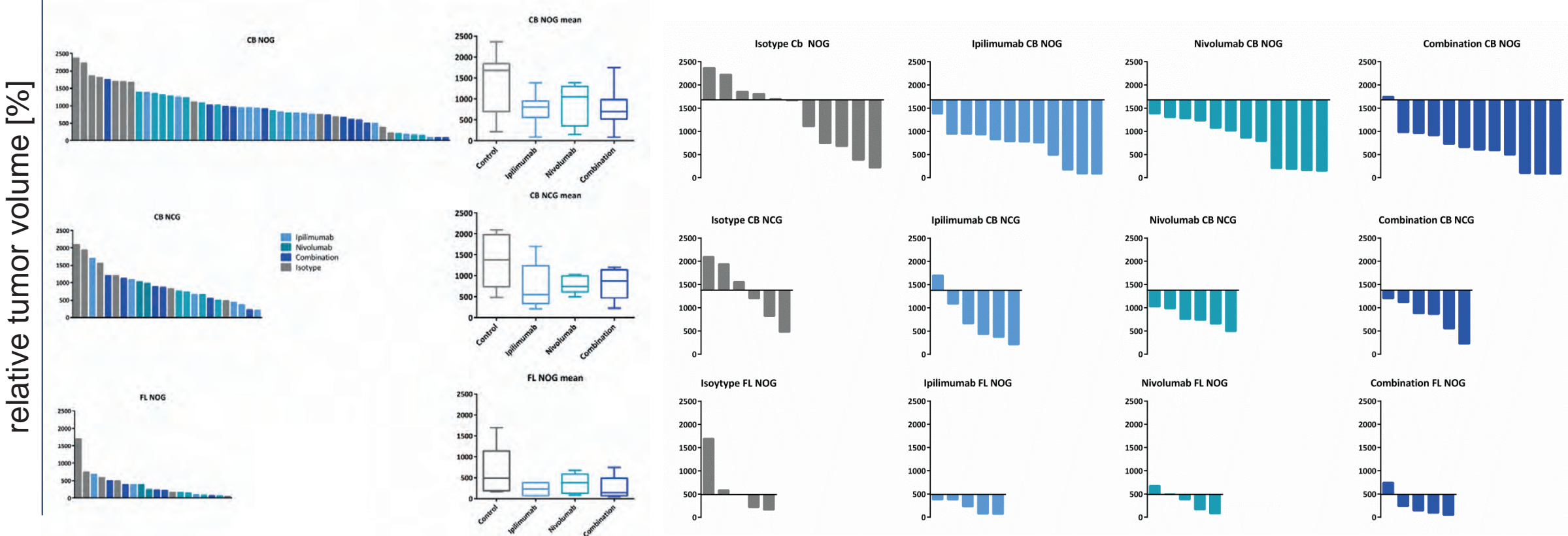


**Fig.10 Detection of huCD45 and huCD3/CD8 cells in PDX tissue by IHC.** Comparison of responder LXFA 1674 vs non-responder LXFE 2324. FC results were confirmed by IHC on tumor tissue. Immune cells are most often localized at the tumor border in the stroma where they often appear in small groups. Numbers of TILs were significantly increased specifically by Ipilimumab treatment.

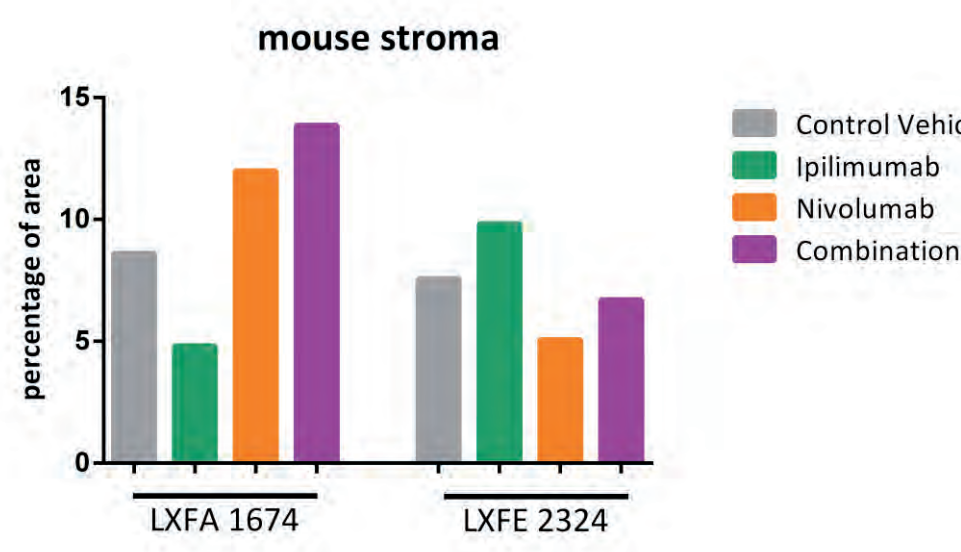


## 2 RESULTS

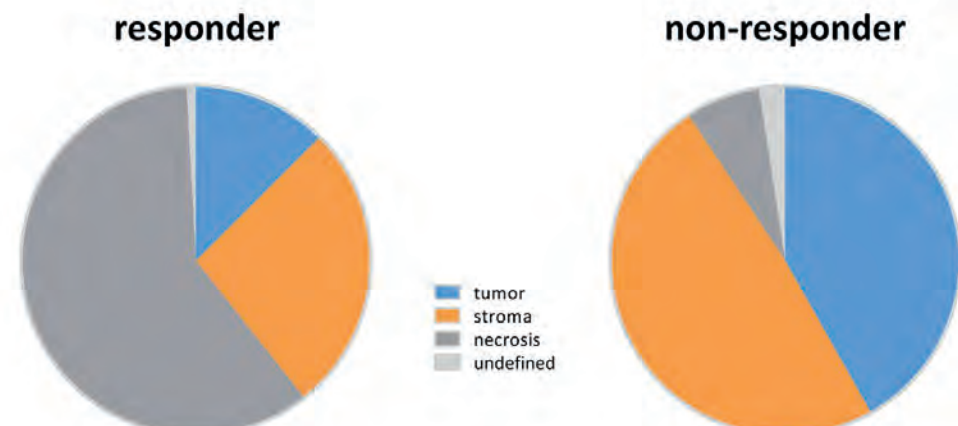
**Fig. 3 Antitumoral activity of two checkpoint inhibitors across twelve PDX models in mono- as well as combined therapy.** Comparison of relative tumor volumes (last ed) between treatment groups. All three treatment regimen displayed a discrete activity pattern throughout the PDX panel which was not influenced by the hosts immunization protocol.



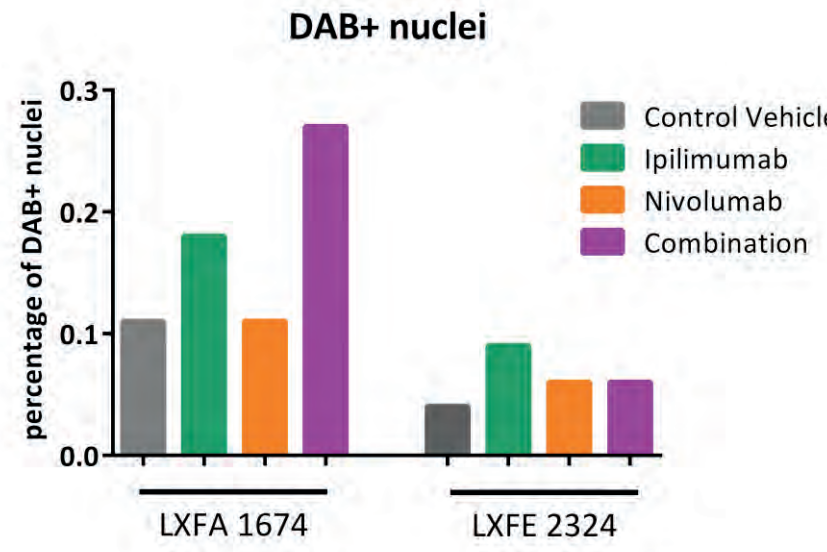
**Fig. 7 Classification of H&E whole-slide digitized images based on morphological image processing techniques.** Whole-slide image analysis of the H&E stains revealed an increase of the stromal compartment proportion in the tumor tissue under treatment with checkpoint inhibitors in responder models. In non-responder models the ratio between tumor and stroma was not influenced by drug treatment.



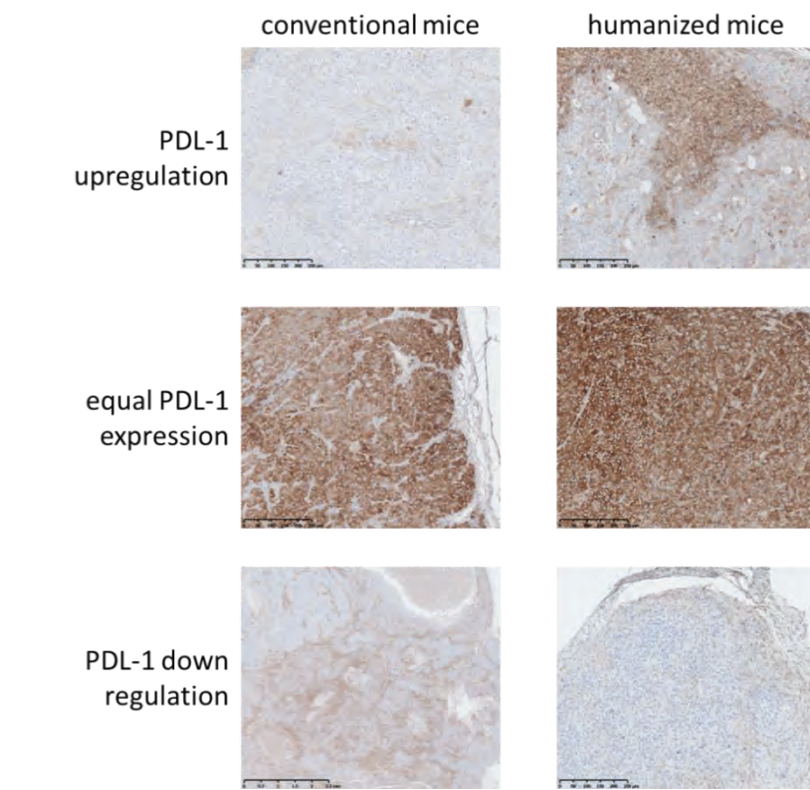
**Fig. 8 Classification of H&E whole-slide digitized images based on morphological image processing techniques.** Whole-slide image analysis of the H&E stains revealed marked differences in the tumor composition between responders and non-responders



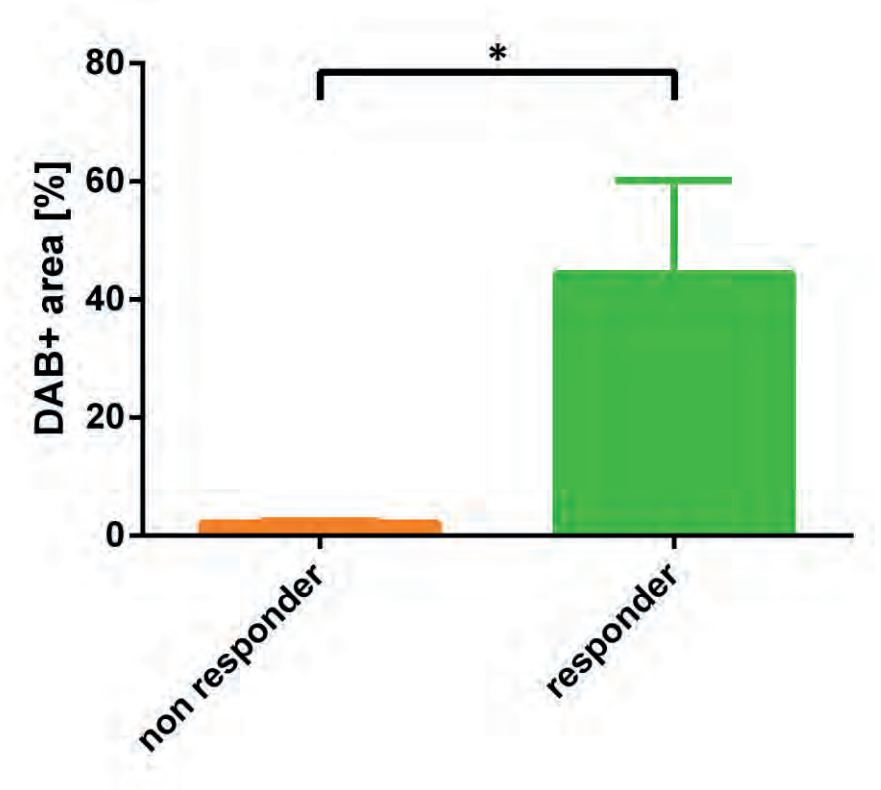
**Fig.9 Detection and quantification of huCD45+ TILs in NSCLC PDX by IHC.** Comparison of responder LXFA 1674 vs non-responder LXFE 2324. PDX models being sensitive towards checkpoint inhibitor treatment (responders) displayed a higher percentage of DAB+ nuclei in huCD45 IHC stains than non-responder models as determined by image analysis. Irrespective thereof, in responders as well as non-responders the treatment with checkpoint inhibitors enhanced the percentage of DAB+ nuclei.



**Fig.11 PDL-1 expression determined by IHC in NSCLC PDX.** PDL-1 expression of the PDX derived tumor cells was influenced by the immune status of the murine hosts. Individual low expressors like LXFA 1674 showed a distinct upregulation. Others, like LXFA 400, (lower panel) showed no upregulation. Models already exhibiting a very high expression in conventional mice (LXFA 1072) depicted no further increase.



**Fig.12 PDL-1 expression determined by IHC in NSCLC PDX.** When dividing individual PDX models into responders and non-responders (cut-off: 30% T/C value). The two groups depicted statistically significant (t-test, two tailed, p value< 0.029) expression levels of PDL-1 determined by IHC:



## 3 CONCLUSIONS

- ✓ The presented in vivo approach combined a reconstituted human immune system and a patient derived xenograft thereby markedly raising its translational significance.
- ✓ 100% of the implanted animals could be assigned to a treatment arm qualifying this study layout for large screening approaches.
- ✓ The study proved the feasibility of the huSMT approach to test I-O compounds e.g. checkpoint inhibitors.
- ✓ A clear link between anti-tumoral activity and possible biomarkers such as T cell infiltration or marker expression could not be drawn.
- ✓ The activity pattern of the twelve PDX mirrored the clinical diversity of tumor responses to checkpoint inhibitor treatment.
- ✓ The combination with a comprehensive image analysis tool enables additional read-outs to quantify anti-tumoral activity of immune modulatory compounds.
- ✓ The latter can be used to identify possible biomarkers in the preclinical setting.
- ✓ Moreover, the translation and validation of these biomarker candidates in a clinical setting is self-evident as primary material needed for these types of analyses is easily accessible.

The use of PDX based humanized mouse models in a SMT format allows screening approaches in complex mouse models. The combination with a comprehensive image analysis tool enables additional read-outs to quantify anti-tumoral activity of immune modulatory compounds. The latter can be used to identify translational biomarker candidates in the preclinical setting.